OMEGA bio-tek

Innovations in nucleic acid isolation

Product Manual

Mag-Bind® Plasmid DNA 96 Kit

M1256-00	1 x 96 preps
M1256-02	24 x 96 preps

September 2013

For research use only.Not intended for diagnostic testing.

Mag-Bind® Plasmid DNA 96 Kit

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Manual Revision: September 2013

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Introduction

The Mag-Bind® family of products is an innovative system that radically simplifies extraction and purification of nucleic acids from a variety of sources. Key to the system is Omega Bio-tek's proprietary Mag-Bind® Particles that avidly, but reversibly, binds DNA or RNA under certain optimal conditions allowing proteins and other contaminants to be removed. Nucleic acids are easily eluted with deionized water or low salt buffer.

The Mag-Bind® Plasmid DNA 96 Kit combines the power of Mag-Bind® technology with the time-tested consistency of alkaline-SDS lysis of bacterial cells to deliver high-quality plasmid DNA. By using a 96-well format, up to 96 samples can be simultaneously processed in less than 60 minutes. The new E-Z 96® Lysate Clearance Plate eliminates time-consuming centrifugation for clearing bacterial alkaline lysates. It also has an average DNA recovery rate 10-30% higher than the manual centrifuge method. Yields vary according to plasmid copy number, *E. coli* strain, and conditions of growth. A 1 mL overnight culture in LB medium typically yields 10-15 µg high-copy plasmid DNA. The purified plasmid DNA can be used directly for automated fluorescent DNA sequencing, as well as for other standard molecular biology techniques including restriction enzyme digestion.

New in this Edition: PFC Buffer replaces isopropanol for binding group of DNA to magnetic beads

Kit Contents

Product Number	M1256-00	M1256-02
Purifications	1 x 96 preps	24 x 96 preps
Mag-Bind® Particles CNR	2.2 mL	50 mL
96-well Microplate (500 µL)	1	24
E-Z 96 Lysate Clearance Plate	1	24
Solution I	30 mL	600 mL
Solution II	30 mL	600 mL
Neutralization Buffer	30 mL	600 mL
Elution Buffer	15 mL	250 mL
PFC Buffer	30 mL	600 mL
EWR Buffer	30 mL	600 mL
PFW Buffer	30 mL	600 mL
SPM Wash Buffer	15 mL	3 x 100 mL
RNase A	100 µL	2 x 1.2 mL
User Manual	✓	✓

Storage and Stability

All Mag-Bind® Plasmid DNA 96 Kit components are guaranteed for at least 12 months from the date of purchase when stored as follows: the mixture of Solution I/RNase A and Mag-Bind® Particles CNR should be stored at 2-8°C; all other materials at 22-25°C.

Preparing Reagents

1. Dilute SPM Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
M1256-00	60 mL
M1256-02	400 mL per bottle

2. Add the vial of RNase A to the bottle of Solution I. Store at 2-8°C.

Guidelines for Vacuum Manifold

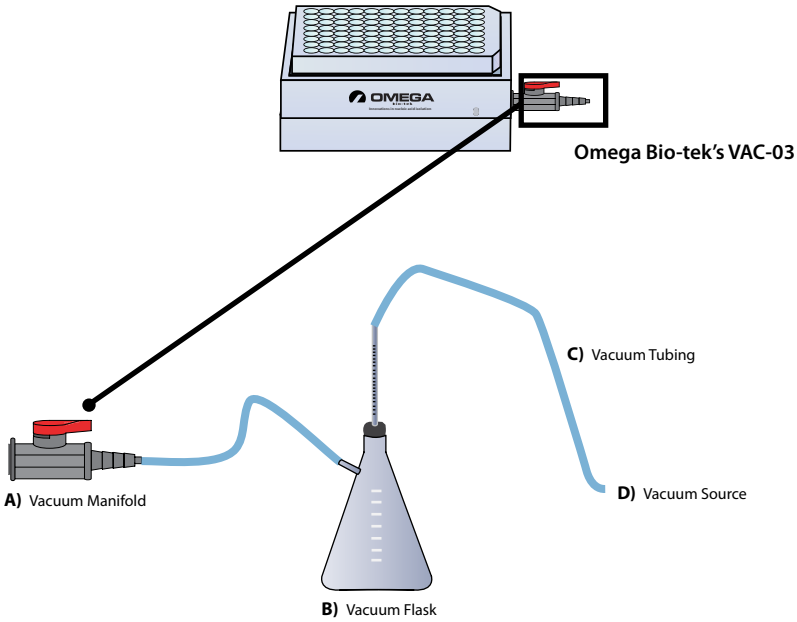
The following is required for use with the Vacuum Protocol:

A) Vacuum Manifold (We recommend Omega Bio-tek's VAC-03)
Other Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma Aldrich VM20, Promega Vacman®, or manifold with standard Luer connector
B) Vacuum Flask
C) Vacuum Tubing
D) Vacuum Source (review tables below for pressure settings)

Manifold	Recommended Pressure (mbar)
VAC-03	-200 to -600

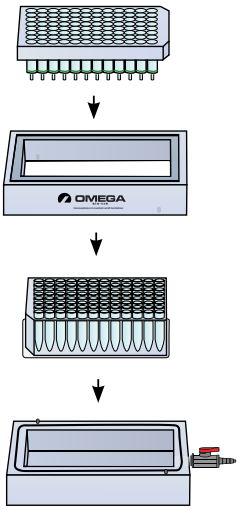
Conversion from millibars:	Multiply by:
millimeters of mercury (mmHg)	0.75
kilopascals (kPa)	0.1
inches of mercury (inHg)	0.0295
Torrs (Torr)	0.75
atmospheres (atm)	0.000987
pounds per square inch (psi)	0.0145

Illustrated Vacuum Setup:



Guidelines for Vacuum Manifold

Lysate Clearance Setup with 1.2 mL 96-well Round-well Plate



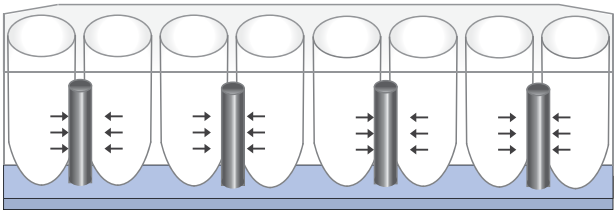
E-Z 96 Lysate Clearance Plate

Vacuum Manifold Top

96-well Round-well Plate (1.2 mL)

Vacuum Manifold Bottom

MSD-01B **Radial Magnet**

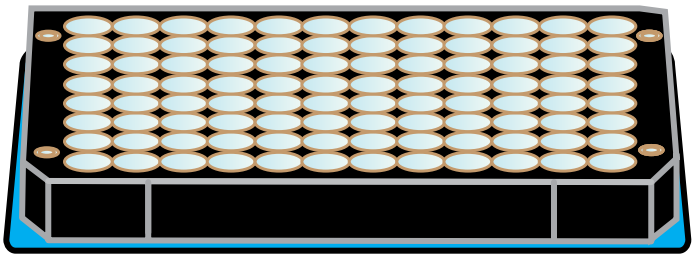


Radial magnet can be used with 1.2 mL or 2.0 mL 96-well deep-well plates where the posts of the magnets can fit in between the wells. The magnetic beads will form a line along the inside of the wells, making it ideal for washing by plate shakers, or for larger volume extractions.

Note: Check the volumes used in the protocol and the speed of plate shaker to ensure no cross contamination will occur.

Alp Aqua 96R Magnetic Stand A001219

Ideal for customers looking to automate the extractions due to an integrated spring that allows for easy and complete supernatant removal without create air suctions which can lead to cross contamination or sample loss. The spring can accomodate up to 1.25 mm of flexibility on the Z axis. Contains 96 individual N48 NdFeB rings for fast magnetization response times of the magnetic beads. Works with both PCR plates, microtiter plates and round bottom deep well plates.
SBS footprint is designed to work with multiple automated liquid handlers and gripper grooves allow for easy placement on and off the magnetic stand. Compatible with Corning Costar 3795, Abgene AB1127 (1.2 mL square well), Abgene AB-0661 (2.2 mL Square well, Nunc 260251 (1.0 mL round well)



Mag-Bind® Plasmid DNA 96 Kit Protocol

Materials and Equipment to be Supplied by User:

- Centrifuge with swinging-bucket rotor at room temperature capable of 4,000 x *g*
- Adapter for 96-well deep-well plates
- Magnetic separation device
- Optional: Incubator capable of 60°C
- Refrigrator capable of 2-8°C
- 100% Ethanol
- Multi-channel pipettor and tips
- Sealing film (Cat# AC1200-01)
- Multi-channel Reservoirs (Cat# AC1331-01)
- 96-well microplates (Cat# EZ9603-01/-02)
- 1.0-2.2 mL 96-well round-well plates capatible with magnetic stand
- Optional: Vacuum pump or vacuum aspirator capable of achieving a vacuum of 20-24 inHg (for vacuum protocol for clearing the cell lysate)
- Optional: Standard vacuum manifold (Cat# VAC-03) (for vacuum protocol for clearing the cell lysate)

Before Starting:

- Prepare SPM Wash Buffer and Solution I/RNase A according to instructions on Page 4.
- Chill Neutralization Buffer to 4°C.
- Vortex the Mag-Bind® Particles CNR thoroughly before use.
- Optional: Heat the Elution Buffer to 60°C.

Note: If you choose to vortex the samples in during the protocol below, make sure to seal the plate completely to avoid any loss of sample or cross-contamination.

1. Grow 1.0-1.5 mL *E. coli* LB cultures in a 2 mL 96-well culture plate at 37°C with agitation with for 16-20 hours.

   Note: It is strongly recommended that an endA negative strain of *E. coli* be used for routine plasmid isolation. Examples of such strains include DH5α® and JM109®.

2. Seal the plate with sealing film.

3. Centrifuge at 3,000 x *g* for 10 minutes at room temperature.

4. Remove the sealing film. Discard supernatant.

5. Dry the plate by placing upside-down on a paper towel to remove excess media.

6. Add 250 µL Solution I. Vortex or pipet up and down to completely resuspend the cell pellet.

   Note: RNase A must be added to Solution I prior to use. Please see Page 4 for instructions.

7. Add 250 µL Solution II. Mix by gently shaking and rotating the plate for 1 minute to obtain a cleared lysate. A 5 minute incubation at room temperature may be necessary.

   Note: Avoid vigorous mixing as doing so will shear chromosomal DNA and lower plasmid purity. Store Solution II tightly capped when not in use.

8. Add 250 µL chilled (4°C) Neutralization Buffer. Mix by gently shaking and rotating the plate for 1 minute until a flocculent white precipitate forms.

9. Choose one of the following methods for lysate clearance:

   A. Clear the cell lysates with centrifugation:
      1. Place the E-Z 96 Lysate Clearance Plate on top of the 96-well Microplate a 1.0-2.2 mL 96-well round-well plate (not provided).
      2. Transfer the lysate from Step 8 to the E-Z 96 Lysate Clearance Plate.
      3. Let sit for 1 minute. The white precipitate should float to the top.
      4. Centrifuge at 2,000 x *g* for 5 minutes.

   B. Clear the cell lysates with vacuum manifold:
      1. Place the 1.2 mL 96-well round-well plate (not provided) into the base of the vacuum manifold. See Page 6 for setup guidelines.
      2. Place the E-Z 96 Lysate Clearance Plate on top of the manifold.
      3. Switch on vacuum source to draw the lysate through the membrane.

10. Add 20 µL Mag-Bind® Particles CNR and 235 µL PFC Buffer to the cleared lysate. Mix thoroughly by pipetting up and down 20 times or shaking.

    Important: The Mag-Bind® Particles CNR will settle and clump together at the bottom of the bottle during storage. Vortex the Mag-Bind® Particles CNR thoroughly before use.

11. Let sit for 12 minutes at room temperature. Mix thoroughly by vortexing or pipetting up and down 20 times.

    Note: For low copy number plasmid isolation a 25 minute or overnight incubation at room temperature may increase yields.

12. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

13. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

14. Remove the plate from the magnetic separation device.

15. Add 250 µL PFW Buffer to each sample.

16. Resuspend the Mag-Bind® Particles CNR by vortexing or pipetting up and down 20 times.

17. Incubate for 1 minute.

18. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

19. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

20. Add 250 µL EWR Wash Buffer to each sample.

21. Resuspend the Mag-Bind® Particles CNR by vortexing or pipetting up and down 20 times.

22. Incubate for 2 minutes.

23. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

24. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

25. Add 250 µL SPM Wash Buffer to each sample.

    Note: SPM Wash Buffer must be diluted with ethanol prior to use. Please see Page 4 for instructions.

26. Resuspend the Mag-Bind® Particles CNR by vortexing or pipetting up and down 20 times.

27. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

28. Aspirate and discard the supernatant. Do not disturb the Mag-Bind® Particles CNR.

29. Leave the plate on the magnetic separation device for 5-10 minutes to air dry the Mag-Bind® Particles CNR. Remove any residual liquid with a pipettor.

30. Remove the plate from the magnetic separation device.

31. Add 50-100 µL Elution Buffer.

32. Resuspend the Mag-Bind® Particles CNR by vortexing or pipetting up and down 20 times.

33. Incubate 2 minutes at room temperature.

    Note: Incubation at 60°C rather than at room temperature may give a modest increase in DNA yield.

34. Place the plate on the magnetic separation device to magnetize the Mag-Bind® Particles CNR. Let sit at room temperature until the Mag-Bind® Particles CNR are completely cleared from solution.

35. Transfer the cleared supernatant containing purified DNA to a clean 96-well microplate (not provided). Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **1-800-832-8896.**

Possible Problems and Suggestions

Problem	Cause	Solution
Low DNA yields	Poor cell lysis	Do not use more than 1 ml with high copy plasmids.
		Cells may not be dispersed adequately prior to addition of Solution I. Vortex cell suspension to completely disperse.
		Increase incubation time with Solution II to obtain a clear lysate
		Solution II if not tightly closed, may need to be replaced.
	Low copy-number plasmid used	Such plasmids may yield as little as 0.1 µg DNA from a 1 ml overnight culture.
	Insufficient EWR Wash Time	EWR wash buffer must be used for a minimum of 120 seconds.
	Insufficient Bind Time	Mag BInd Particles CNR, Lysate and PFC buffer must be mixed and incubated for a minimum of 10 minutes
Problem	Cause	Solution
No DNA eluted	SPM Wash Buffer is not diluted with ethanol.	Prepare SPM Wash Buffer as instructed on the label.
High-molecular weight DNA contamination	Over mixing of cell lysate upon addition of Solution II	Do not vortex or aggressively mix after adding Solution II. Simply inverting and rotating tube to cover walls with viscous lysate.
Optical densities do not agree with DNA yield on agarose gel	race contaminants eluted from column increase A260	Make sure to wash Mag-Bind® pellet as instructed. Alternatively, rely on agarose gel/ ethidium bromide electrophoresis or dye based method for quantification.
RNA visible on agarose gel	RNase A not added to Solution I	Add 1 vial of RNase to each bottle of Solution I.
DNA floats out of well while loading agarose gel	Ethanol not completely removed before elution	Increase air dry time before elution step
DNA will not perform in downstream applications	Traces of ethanol remain on column prior to elution	The DNA plate must be washed with absolute ethanol and dried before elution. Ethanol precipitation may be required following elution.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
Alp Aqua Magnetic Stand 96R	A001322
E-Z 96 Magnetic Separation Device, Radial Magnetizing	MSD-01B
E-Z 96 Vacuum Manifold	VAC-03
Multi-Channel Disposable Reservoirs, 100/pk	AC1331-01
SealPlate Film, 100/box	AC1200-01
96-well Microplate (500 µL), 5/pk	EZ9604-01
96-well Microplate (500 µL), 25/pk	EZ9604-02
Solution I, 250 mL	PS001
Solution II, 250 mL	PS002
Neutralization Buffer, 250 mL	PS004
SPM Wash Buffer, 40 mL	PS014
Elution Buffer, 100 mL	PDR048
RNase A, 400 µL	AC117
RNase A, 5 mL	AC118

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